REMARKS

Claims 1, 4-12, and 15-23 are presently pending. Of these, Claims 8-11 are withdrawn from consideration. Support for amendments to the claims is discussed below. No new matter has been added herewith. The following addresses the substance of the Office Action.

Abstract

The Examiner indicated that the application does not contain an abstract of the disclosure as required by 37 C.F.R. § 1.72(b). The Applicants note that an Abstract was added by way of Preliminary Amendment dated August 10, 2006. Accordingly, the application is believed to meet the requirements with regard to an abstract.

Claim Objections

Claims 4, 7, 12 and 12 were objected to for reciting "TEM-1" without providing the full recitation of the abbreviation. Referring to Heritage J. et al. (1999 *J Antimicrobial Chemotherapy* **44**:309-318; reference submitted herewith with an Information Disclosure Statement), at page 310, first column, lines 5-7, the nomenclature "TEM" is based on "Temoniera," the name of a patient from whom resistant bacteria were isolated. Accordingly, "TEM-1" is not an acronym that has an equivalent full recitation. As such, recitation of TEM-1 is believed to be proper.

Indefiniteness Under 35 U.S.C. § 112, Second Paragraph

Claim 6 was rejected because the Examiner indicated that the phrase "derived from" is not clear. The phrase is amended to recite "wherein the β-lactamase moiety is a class A β-lactamase." Withdrawn Claims 8 and 10 have also been amended in the same way. Accordingly, Claim 6, 8 and 10 are in compliance with 35 U.S.C. § 112, second paragraph.

Claims 21 and 23 were rejected because the claims recite "hybridize under stringent conditions." The Claims did not recite conditions under which the hybridization must occur. Applicants refer to Ausubel, F.M. et al. in Current Protocols in Molecular Biology, John Wiley & Sons, Inc. 1987-1994 and 1994-2009, Section 2.10, pages 1-16; copy submitted herewith along with an Information Disclosure Statement), which shows that one of ordinary skill in the art would know what is meant by stringent conditions. In particular, referring to page 2.10.3 of Ausubel et al. defines high stringency as 0.1x SSC and 0.1% SDS at 68°C. One of skill ordinary skill in the art would interpret "under stringent conditions" in light of commonly know reference

manuals, such as Ausubel et al. to mean under conditions of high stringency (i.e., 0.1x SSC and 0.1% SDS at 68°C). As such, the claims are in compliance with 35 U.S.C. § 112, Second Paragraph.

Enablement Under 35 U.S.C. § 112, First Paragraph

Claims 1, 4-7, 12 and 15-23 were rejected because the Examiner stated that the Specification does not reasonably provide enablement for any polynucleotide sequence encoding a bifunctional hybrid protein wherein said bifunctional hybrid protein comprises: i) any or all β-lactamase and its variants, mutants and recombinants of undefined structure from any or all sources; ii) encoded by any polynucleotide sequence of SEQ ID NO: 2 of any length (fragments) and its variants of undefined structure hybridizing under any undefined stringent conditions to SEQ 1D NO: 2; iii) said β-lactamase protein of undefined structure bearing a heterologous protein located between any two neighboring alpha helices and having biological function and further said heterologous protein encoded by polynucleotide sequence of SEQ ID NO: 25 of any length (fragments) and its variants of undefined structure hybridizing under any undefined stringent conditions to SEQ ID NO: 25 and having protein A activity or biological function.

The Applicants assert that the Specification enables claims that encompass multiple β-lactamase carrier proteins that have different heterologous sequences inserted between alpha helices 8 and 9. In particular, the Applicants wish to point out to the Examiner that the specification exemplifies that 1) different β-lactamase proteins function as carriers for inserts positioned between helices 8 and 9; and 2) different heterologous sequences maintain activity when inserted between helices 8 and 9 of the various types of β-lactamase proteins. The following Table 1 summarizes the results of Examples 1-20.

Table 1. Experimental Data in the Examples of the Specification

Hybrid β-lactamase/heterologous sequence	Example(s)	Activity		Paragraph in Specification Indicating Dual Activities
		β-Lac	insert	
TEM-1/Sta	2-9	Yes	Yes	[00112]
TEM-1/Protein A	10	Yes	Yes	[00119]

BlaP/Protein A	11	Yes	Yes	[00122]
TEM-1/Protein G	12	Yes	Yes	[00127]
BlaP/Protein G	13	Yes	Yes	[00129]
BlaP/HA	14	Yes	Yes	[00134]
TEM-1/PLA_2	15	Yes	Yes	[00136]
BlaP/LPS	16	Yes	N.D.	[00138]
AmpC/Protein A	18	Yes	Yes	[00147]
BlaR-CTD/Hemagglutinin	19	Yes	Yes	[00151]
BlaR-CTD/Protein A	20	Yes	Yes	[00153]

*ND: Not determined

In summary, the specification presents eleven different combinations of hybrid β-lactamase/heterologous sequences, wherein numerous β-lactamases are combined with various heterologous inserts. Given the breadth of scope represented by these examples, the specification is enabling for one of ordinary skill in the art to practice the full scope of Claim 1.

Moreover, a declaration under 37 C.F.R. § 1.132 by Dr. Fabrizio Giannotta, Ph.D. is submitted herewith to further explain the enablement provided by the present specification. The declaration provides full methodological details relating to different β-lactamase proteins, which functioned as carriers for inserts positioned between helices 8 and 9 are disclosed in the present Specification. The graphs, figures and tables provided in the Specification present data that were obtained with such proteins (with the sole exception of Example 16). Moreover, numerous scientific publications, which are based on the presently claimed recombinant nucleotide sequences that encode bifunctional hybrid active-site serine β-lactamase proteins, show that various β-lactamases function as carriers for inserts positioned between helices 8 and 9 as disclosed in the present Specification. As summarized at item 5d of Dr. Giannotta' declaration, such publications include:

Ruth N. et al. 2005 Vaccine 23:3618-3627;

Ruth N. et al. 2008 FEBS J 275:5150-5160;

Vandevenne, M. et al. 2008 Prot Eng Des Sel 21:443-51;

Vandevenne, M. et al. 2007 Prot Sci 16:2260-2271; and

Chevigne, A. et al. 2007 J Immunol Methods 320:81-93.

Copies of the five above-referenced publications are submitted herewith.

Ruth et al. (The FEBS Journal, 2008) describes the insertion of different homologous sequences into different sites of TEM-1 beta-lactamase: 37, 195, 198, 206, 216, 218, 232 and 260. Accordingly, this publication supports enablement of the present claims.

Chevigne et al. (Journal of Immunological Methods, 2007) refers to BlaP beta-lactamase hybrid proteins and on page 82, left col., 1st full paragraph to page 83, left col. The adavantages of the construct and the method of construction are explained (namely insertion in the TEM-1 corresponding loop of the BlaP beta-lactamase).

Vandevenne et al. (Protein Science, 2007) also describes BlaP beta-lactamase hybrid proteins having a 73 amino acid residue heterologous sequence inserted in the loop connecting helices 9 and 10, namely between Asp (D) 197 and Lys (K) 198 (see page 2261, left col., last 3 lines and right col.. The residues D and K are the same and are located always in the loop connecting helices 9 and 10.

Vandevenne et al. (PEDS, Protein Engineering, Design & Selection, 2008) also describes BlaP beta-lactamase hybrid proteins having heterologous sequence inserted in the loop connecting helices 9 and 10, namely between Asp (D) 211 and Lys (K) 212 (see page 3 of 9, section "Results", Abstract and page 1 of 9, right column).

In view of the data presented in the Specification and in the above-referenced scientific publications, the skilled artisan could readily make and use the full scope of the claimed invention without undue experimentation. Therefore, the claims are in compliance with the enablement requirement of 35 U.S.C. § 112, first paragraph.

Written Description Under 35 U.S.C. § 112, First Paragraph

Claims 1, 4-7, 12 and 15-23 were rejected because they were interpreted as being directed to any polynucleotide sequence as defined above in the previous section regarding enablement. In particular, the Examiner stated that the claims encompass a genus of structures without any structural limitation and that the specification is silent in regard to (1) the structures of all the polynucleotides and the encoded polypeptides encompassed by the claims, and (2) the critical

structural elements of any variant, mutant or recombinant β-lactamase protein from any source and having associated function.

The Applicants wish to point out that one of ordinary skill in the art understands that classes A, C and D of serine active β-lactamases share a common three-dimensional structure, which is characterized by two domains (i.e., an α-helix domain and a domain containing α-helices and β-sheets. Consequently, the structure of the scaffold is well known, characterized and restricted to serine active β-lactamases. Moreover, the Applicants have amended the claims to recite that the at least one heterologous sequence is inserted in a region forming a juncture between alpha helix 8 and alpha helix 9 of said active-site serine β-lactamase.

The skilled artisan would also recognize that the polynucleotide sequence inserted within the β-lactamase scaffold may vary with regard to length, structure or function. In view of the highly conserved structural domains of the active-site serine β-lactamase proteins and the amendments to the claims, the skilled artisan could readily recognize that the Applicants were in full possession of the invention as presently claimed. Accordingly, the Applicants respectfully request that the Examiner reconsider and withdrawal the rejection under 35 U.S.C. § 112, first Paragraph.

Obviousness under 35 U.S.C. § 103(a)

Claims 1, 4-7, 12 and 15-23 were rejected as being unpatentable over Murray et al. (WO 03/105753) in view of Balint et al. (U.S. Application Publication No. 2003/0165825 A1) Galarneau et al. (*Nature Biotechnology* 2002 **20**:619-622) Neugebauer et al. (*Nucleic Acids Res* 1981 **9**:2577-2588) and Finck-Barbancon et al. (*FEMS Microbiol Lett* 1992 **70**:1-8).

However, as explained below, even if one having ordinary skill in the art were to combine the references in the manner suggested by the Examiner, significant unexpected advantages would be obtained. One of ordinary skill in the art could in no way have predicted these unexpected properties of the resulting invention. Accordingly, these unexpected advantages would rebut any *prima facie* showing of obviousness resulting from the cited combination of references. Moreover, as also explained below, no *prima facie* showing of obviousness can be created by the cited combination of references because one having ordinary skill in the art would not have combined the references in the manner suggested by the Examiner.

Unexpected Advantages

The Applicants point out that unexpected results are obtained when a heterologous sequence is inserted between helices 8 and 9, as opposed to insertion at other sites. In this regard, the Applicants note that paragraph [00112] states that TEM-1 β-lactamase allowed the insertion of large peptide sequences between the helices α8 and α9 (position 197) and also between helices α9 and α10 (site 216) without any major loss of activity and stability. Ruth et al. (The FEBS Journal, 2008) describes the insertion of different homologous sequences into different sites of TEM-1 beta-lactamase: 37, 195, 198, 206, 216, 218, 232 and 260. Referring to page 7, left col., line 2 to right col., line 29 the authors summarize that the loop between helix 8 and 9 (insertion sites 195 and 198) are the most suitable insertion sites, even better than the insertion site 216 and 218. This was an unexpected result and one of ordinary skill in the art would not have expected that the loop between helix 8 and 9 would be a better point of insertion than the loop between helices α9 and α10.

Comments Concerning the Combination of References Cited

With regard to the cited references, Murray et al. relates to targeted enzymes that bind to targets, such as cancer cells or cancer cell markers, better than the corresponding pre-targeted enzymes. Such targeted enzymes have a variant sequence that enhances binding to a target while maintaining enzymatic function. Unlike the presently claimed compositions, the variant sequences described by Murray et al. can be placed anywhere in the structure of the pre-targeted enzyme (see Murray et al., page 55, lines 14-15). In addition, there is no need for heterologous sequence to have a biological function that is different from the β-lactamase enzyme.

Balint et al. and Galarneau et al. relate to interaction-activated protein fragments, wherein protein domain fragments become active by virtue of specific interaction between the fragments. Balint et al. teaches break point termini for β-lactamase, wherein subdomain fragments, which are defined by such break point termini, can be conjugated together by a heterologous domain that brings the sub-domains together to achieve interaction-dependent fragment complementation by the sub-domains. However, Balint defines the insertion point as the region between helices 7 and 8. Moreover, unlike the presently claimed compositions, it is not necessary for the heterologous domain to have a biological function that is different from the β-lactamase enzyme. Similarly, Galarneau et al. teaches fragment complementation by fragments of β-lactamase

enzymes, without requiring that there be a heterologous sequence, let alone a requirement that it have a biological function that is different from the β-lactamase enzyme.

In contrast to the combined disclosures of Murray et al., Balint et al., and Galarneau et al., a technological advance offered by the present invention involves internalizing a heterologous sequence within the native structure of a carrier protein (i.e., an active-site serine β-lactamase). This new approach provides a means of replacing the internalized fragments in a 3-dimensional context that approximates the native background of the heterologous sequence. The constraints imposed by the carrier protein compel the internalized peptides to adopt a proper structure that results in biological activity by the heterologous sequence. The outcome is the creation of a single-polypeptide, bifunctional hybrid β-lactamase that contains biologically active heterologous sequence.

The heterologous sequence internalized in the context of the last two alpha helices before the alpha/beta domain of the β-lactamase sequence is able to adopt a folding close to its native conformation, thereby retaining its biological activity. This specific feature results in a hybrid bifunctional protein where a specific and effective enzymatic activity is associated with the biochemical properties of the heterologous sequence.

Transposition and phage display experiments have shown the possibility for introducing or degenerating very short sequences of nucleotides (8 to 30 nucleotides) in the coding sequence for the TEM-1 β-lactamase and using these mutated genes as a basis for synthesizing a constantly functional enzyme. In contrast, the idea of the present invention is not to create a chimera bank that theoretically covers all types of biodiversity. Instead, the presently claimed recombinant nucleotide sequences favor insertion of large peptide sequences present in the heterologous sequence, which has biochemical characteristics that are already identified. Consequently, the present invention provides a means of internalizing a peptide sequence that has already been naturally optimized for a given property and averting (as is the case with the phage display technique) evolutionary mutagenesis reactions within the insert and carrier protein. With the presently claimed system, chimera recombinant nucleotides may be restricted to a few thousand clones (or a few dozens) such that the screening is quicker and more targeted.

Presentation of the heterologous sequence by the beta-lactamase carrier involves advantages that were neither possible nor appreciated by the prior art. For example, the heterologous polypeptide is displayed within a protein environment, which mimics wild-type

protein constraints. It is often difficult to achieve the same feature with fused peptides, synthetic peptides or chemical conjugations. Due to the fact that the presently claimed technology involves a gene insertion, as opposed to a fusion, the folding of both the exogenous polypeptide and the enzyme scaffold are strongly inter-dependent. Consequently, the correct folding of the enzyme scaffold, and hence its biological activity, is conditioned by the correct folding of the exogenous polypeptide of interest. Given the antibiotic resistance activity of β-lactamase, the cloning procedure is very efficient, resulting in greater than 99% of in-frame insertion by the heterologous sequence. β-lactamase constructs with heterologous inserts produce a full-length, active enzyme that protects bacteria against the lethal effect of ampicillin. With this procedure, heterologous sequences that are not in frame or are in reverse orientation lack β-lactamase activity. Moreover, expression and production of the heterologous protein domain is enhanced by the soluble carrier.

Based on the cited references one of ordinary skill in the art would have had no reason to develop the presently claimed constructs, wherein a heterologous sequence is inserted in a specified location within the β-lactamase coding sequence, resulting in a construct that encodes a single-polypeptide, bifunctional hybrid β-lactamase that has the biological activities of both β-lactamase and the inserted sequence. Such constructs were not envisioned by the prior art, and they have advantages over chimeric β-lactamase proteins or complementary fragments disclosed in the cited references. Accordingly, the claims are not obvious and the rejection under 35 U.S.C. § 103(a) should be withdrawn.

No Disclaimers or Disavowals

Although the present communication may include alterations to the application or claims, or characterizations of claim scope or referenced art, Applicant is not conceding in this application that previously pending claims are not patentable over the cited references. Rather, any alterations or characterizations are being made to facilitate expeditious prosecution of this application. Applicant reserves the right to pursue at a later date any previously pending or other broader or narrower claims that capture any subject matter supported by the present disclosure, including subject matter found to be specifically disclaimed herein or by any prior prosecution. Accordingly, reviewers of this or any parent, child or related prosecution history shall not reasonably infer that Applicant has made any disclaimers or disavowals of any subject matter supported by the present application.

CONCLUSION

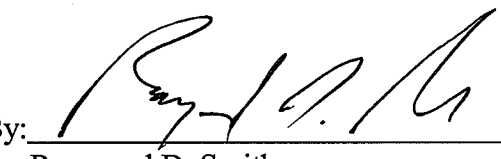
In view of Applicants' amendments to the Specification and the Claims and the foregoing Remarks, it is respectfully submitted that the present application is in condition for allowance. Should the Examiner have any remaining concerns which might prevent the prompt allowance of the application, the Examiner is respectfully invited to contact the undersigned at the telephone number appearing below.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: October 22, 2009

By: 

Raymond D. Smith
Registration No. 55,634
Agent of Record
Customer No. 20995
(949) 760-0404

7991594
102109